AD____________________

Award Number DAMD17-01-1-0830

TITLE: Selective Cytotoxic Phospholipids for Prostate Cancer

PRINCIPAL INVESTIGATOR: Duane D. Miller, Ph.D.

CONTRACTING ORGANIZATION: The University of Tennessee
Memphis, Tennessee 38163

REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Annual (20 September 2001 - 19 September 2002)

4. TITLE AND SUBTITLE

Selective Cytotoxic Phospholipids for Prostate Cancer

5. FUNDING NUNUMBER

DAMD17-01-1-0830

6. AUTHOR(S)

Duane D. Miller, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Tennessee
Memphis, Tennessee 38163

email **dmiller@utmem.edu**

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The goal of this project is to build upon our discovery of two phospholipid lead compounds, serine amide phosphate **(SAP)** and serine diamide phosphate **(SDAP)**, that have been shown to be selective in their cytotoxic actions in PC-3 and DU-145 prostate caner cells respectively. These agents were originally desingned as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to snythesize a focused set of SAP and SDAP analogs. We have found that the synthesis of these compounds can be prepared in a shorter sequence and in better yield our new sythetic process outlined in Scheme 3. We have tested for the affinity of the synthesized compounds in PC-3, DU-145, and LNCaP cell lines as we proposed earlier. In addition to these cell lines we have also tested for affinity of these compounds in two additional PPC-1 and TSU cell lines (data shown in Table 1). These new analogs have provided valuable insight as to the importance of chirality, lipid solubility, spatial orientation, and important functional groups of the pharamcophore and for the optimization of the antiproliferative actions of this new set of drugs. One of the key factors is that the Serine Amides and the N-BOC-Serine Amide alcohol series which lack a phosphate show higher activity with longer aliphatic chains. We have not found the optimum length of the aliphatic chain in these two series. In earliar studies it appeared in our Serine Amide Phosphate (SAP) series that the alphatic chain is optimum at C-14 on DU-145 and PC-3 cell lines. However, the pure isomers do not show any large differences and the activity is different from earlier tests run on the racemic mixtures. We have observed that one of the isomers of the SAP series with the aliphatic chain being C-18 does have moderate acitivity against all of the cancer cell lines. Again it appears that we should make and test new SAP series with longer aliphatic chains and investigate the effects of unsaturation.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Phospholipid, cytotoxic, Lysophosphatidic acid, antiproliferation, Chirality, pharmacophore, lipid solubility, synthesis,	11
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT Unlimited
Unclassified	Unclassified	Unclassified	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Introduction The goal of this project is to build upon our discovery of two phospholipid lead compounds, serine amide phosphate (**SAP**) and serine diamide phosphate (**SDAP**), that have been shown to be selective in their cytotoxic actions in PC-3 and DU-145 prostate caner cells respectively. These agents were originally desingned as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to snythesize a focused set of SAP and SDAP analogs using the combinatorial parallel-compound solution phase synthesies when appropiate, and to prepare the remaining analogs using classical techniques. These new analogs will provide valuable insight as to the importance of chirality, lipid solubility, spatial orientation, and important functional groups of the pharamcophore and allow for the optimization of the antiproliferative actions of this new set of drugs. We have developed several new synthetic schemes and are now utilizing the procedures outlined in Scheme 3 of our report.

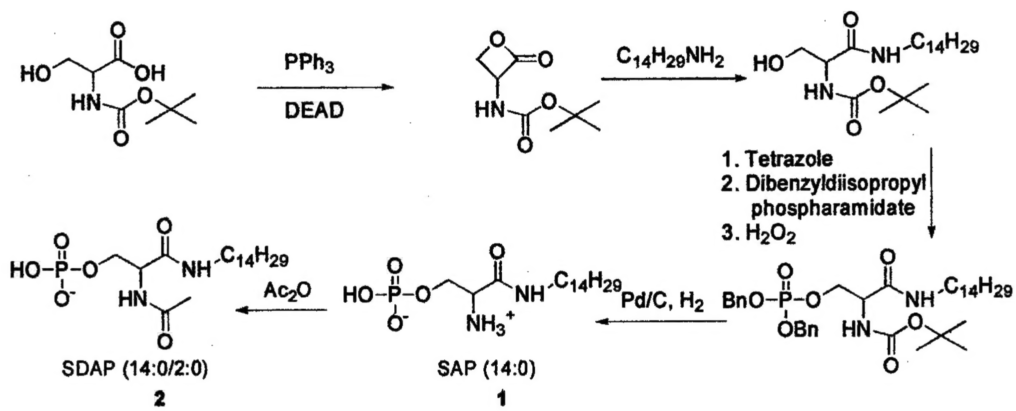
Once we have found some compoundscompounds that display the most potent in vitro anti-proliferative activity, in vivo studies in tumor-bearing nude ice will be initiated. Due to time and budgetary constraints, only our two most promising compounds (i.e., those with the lowest IC50 values during in vitro studies) will be carried forward to these studies in the next year of the grant. These experiments are designed to provide an initial pharmacologic assessment of our most promising compounds, focusing specifically on (1) their in vivo toxicity and (2) their in vivo antitumor efficacy in prostate tumor xenografts. Animal care guidelines at our institution will be strictly followed for these studies.

Task 1. Synthesis of serine amide phosphate (SAP) and serine diamide phosphate (SDAP) analogs

Year 1: We will prepare R and S optical isomers of SAP and SDAP to check for the importance of chirality.

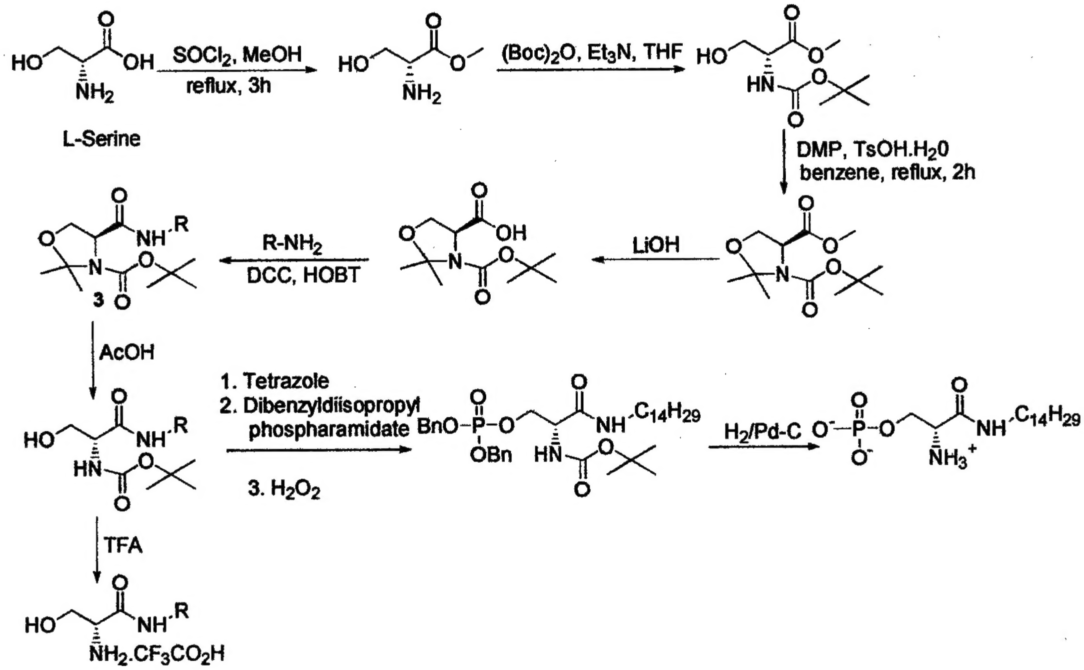
This task was successfully completed. The *R* and *S* optical isomers of SAP (14:0) have been synthesized by an improved method. The active compounds SAP (14:0, **1**) and SDAP (14:0/2:0, **2**) were synthesized following Scheme 1. In this synthetic sequence it was difficult to handle chemicals like DEAD and PPh_3. The reaction yields were very low at key β–lactone formation and amide bond formation steps. This scheme may also potentially give racemic products when nucleophilic amine opens the β-lactone ring.

SCHEME 1



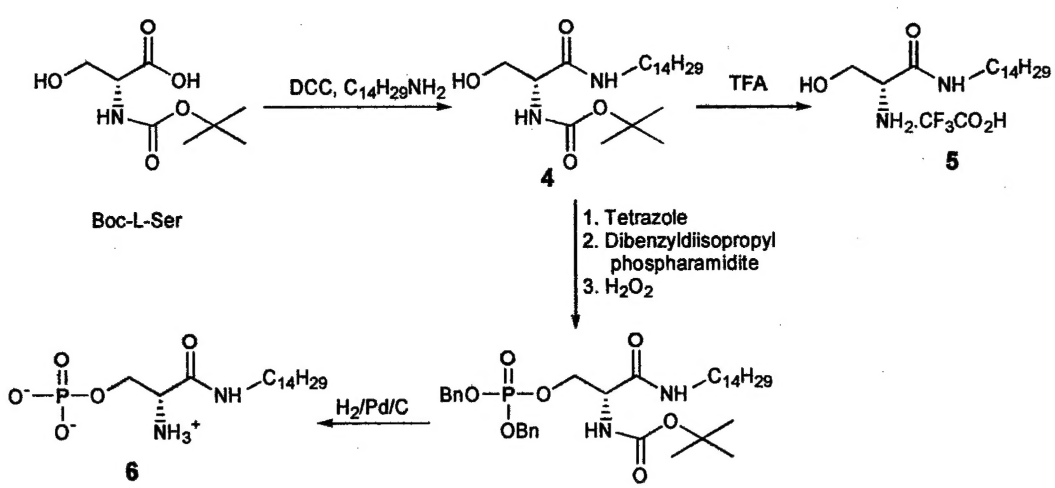
To overcome the low yields and to avoid possible racemization we proposed a new synthetic route to get optically pure SAPs as shown in Scheme 2. In this method though we protect the chirality of the starting serine through out the synthesis, the deprotection of the acetonide intermediate (**3**) to give the free alcohol was unsuccessful under a host of reaction conditions. On the other hand Scheme 2 has too many steps involved to get to the final product.

SCHEME 2



In due process we have changed our synthetic sequence to Scheme 3, which was very efficient with fewer steps and high yields. Initially we used DCC/HOBT as coupling reagent for the amide bond formation, which gave us problems at purifications. So, we tried several coupling reagents like PyBOP, BOP, and EDC to optimize the key step in the synthetic scheme. EDC is a water-soluble coupling reagent and worked well for amide bond formation in our scheme. With this reagent there is no need of any further purification steps since it gives considerably pure product to take it to the next step in the synthesis.

SCHEME 3

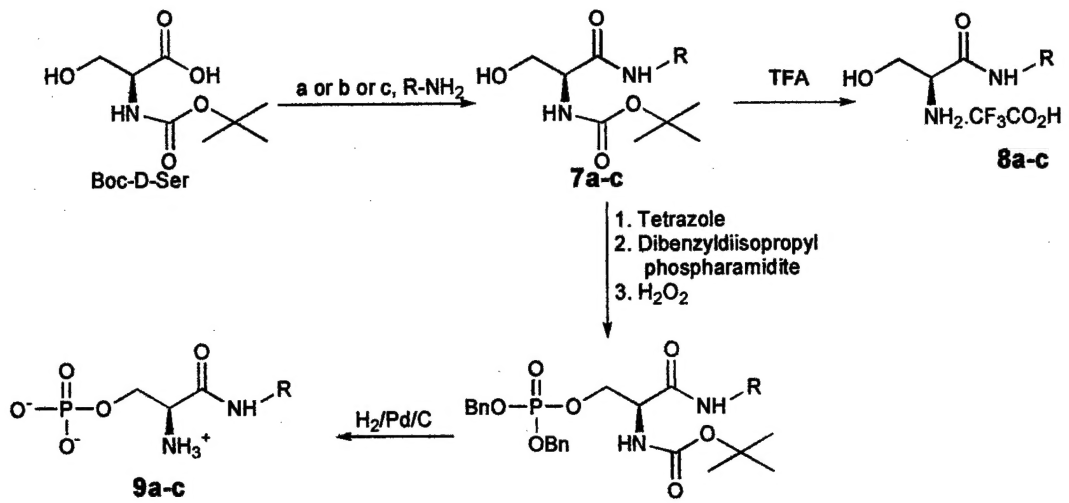


We will prepare the aliphatic chain variations of SAP and SDAP in order to optimize the lipid solubility of this new set of drugs.

We will determine the correct structure of new analogs by NMR, CMR, UV, IR and Mass Spectrometry.

These tasks were successfully completed. Following our new synthetic protocol (as described for the synthesis of SAP (14:0)). We have completed the synthesis of 10:0, 14:0, 18:0, and 19:0 D-SAPs shown in scheme 4. The structures of all new compounds synthesized were confirmed using elemental analysis and spectroscopic data (1H, 13C, IR and Mass Spectrometry).

SCHEME 4[a]



[a] Reagents: (a) DCC, $C_{14}H_{29}NH_2$, HOBT. (b) PyBOP, DIEA. (c) EDC, DMAP

	R
7a, 8a, 9a	$C_{10}H_{21}$
7b, 8b, 9b	$C_{14}H_{29}$
7c, 8c, 9c	$C_{18}H_{37}$

<u>Task 2. Determine activity of SAP and SDAP analogs in Prostate cell lines</u>

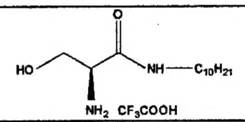
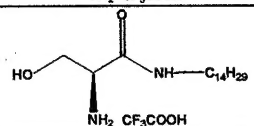
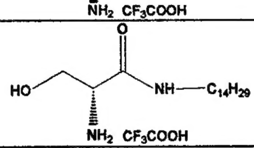
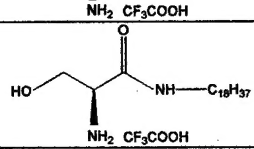
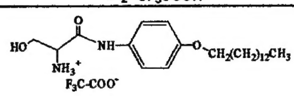
Year 1: We will determine the activity of the synthesized analogs in Specific Aims 1.2.3 in PC-3, DU-145 and LNCaP cell lines.

This task was completed successfully. We have tested for the affinity of the synthesized compounds in PC-3, DU-145, and LNCaP cell lines as we proposed earlier. In addition to these cell lines we have also tested for affinity of these compounds in two additional PPC-1 and TSU cell lines (data shown in Table 1)

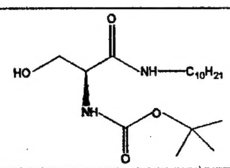
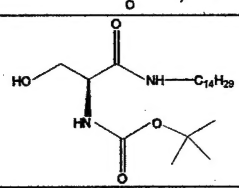
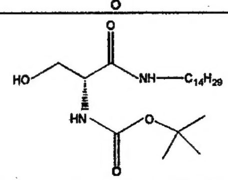
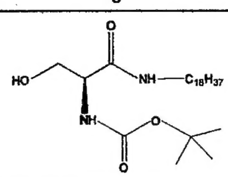
Early experiments were performed with VMS-3-XXX series (racemic mixtures) and S-3-11-B (racemic mixture) in DU-145, PC3, and LNCaP cells in 2000. SH-I-XX and GD-1-

XX compounds were tested in DU-145, PC3, LNCaP, PPC-1, and TSU cells in June-July, 2002.

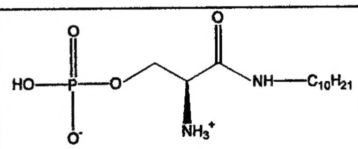
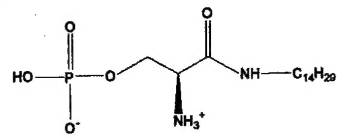
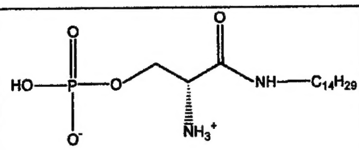
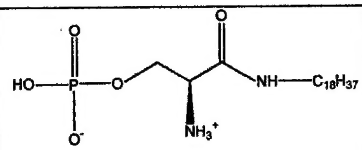
1. Serine amides

Compound ID	Structure	IC_{50} (μM)				
		DU-145	PC3	LNCaP	PPC-1	TSU
7b (SH-I-67)		52.2	35.0	31.0	15.9	26.0
8b (SH-I-33)		8.2	10.2	8.1	6.3	7.6
5 (GD-1-45)		6.9	10.3	10.0	6.2	9.2
9b (SH-I-69)		5.4	5.2	3.8	2.2	4.4
VMS-3-119 (racemic mixture)		38.9	> 50	> 50	Not tested	Not tested

2. N-Boc-Serine amides

Compound ID	Structure	IC_{50} (μM)				
		DU-145	PC3	LNCaP	PPC-1	TSU
7a (SH-I-55)		21.0	23.0	15.0	17.7	4.4*
S-3-11-B (racemic mixture)		19.7	> 50	10.9	Not tested	Not tested
8a (SH-I-11)		12.3	13.1	10.9	19.7	10.0
4 (GD-1-28)		21.4	16.6	13.6	14.3	12.5
9a (SH-I-49)		7.9	7.5	10.3	7.9	2.4

3. Serine amide phosphates

Compound ID	Structure	IC50 (μM)				
		DU-145	PC3	LNCaP	PPC-1	TSU
VMS-3-175 (racemic mixture)		24.9	31.6	4.9	Not tested	Not tested
7c (SH-I-65)		50.2	36.0	44.7	22.1	31.5
VMS-3-159 [a] (racemic mixture)		2.3	0.7	13.5	Not tested	Not tested
8c (SH-I-31)[b]		20.6	> 100	10.1	> 10	> 10
6 (GD-1-43)		32.0	> 200	19.7	~ 10	~ 10
VMS-3-173 (racemic mixture)		9.1	> 50	10.7	Not tested	Not tested
9c (SH-I-59)		11.7	5.7*	4.4*	3.2*	4.8

[a] 1 mM stock solution in acidic methanol tested in March, 2000.
IC_{50}'s were higher than 10 μM in all three cell lines when tested with solid drug later in 2000.

[b] Powder tested in June, 2002.

	IC50				
	DU-145	PC3	LNCaP	PPC-1	TSU
5-FU (μM)	11.9	12.0	4.9	6.4	3.6
Doxorubicin (nM)	15.6	52.8	13.4	12.0	15.1
Paclitaxel (nM)	2.7	3.4	2.0	3.4	2.1

<u>Task 3. Determine the activity of SAP and SDAP analogs in prostate tumor xenpgrafts in mice</u>

Year 1: We will select the most promising agents from Specific Aim 6 of the PC-3, DU-145 and LNCaP cell lines studies for In Vivo Efficacy against Prostate Tumor Xenografts in mice (Specific Aim C.7).

We are still optimizing the compounds for the optimum activity in PC-3, DU-145, LNCaP, PPC-1 and TSU cell lines. Once we get the most promising agents in In Vitro analyses we will test for their In vivo efficacy against prostate tumor xenografts in mice.

Key Research Accomplishments

- Developed a short and efficient new method for the synthesis of *R* and *S* isomers of SAP & SDAP analogs in high optical purity.
- Synthesized and fully characterized novel serine amide phosphates using elemental analyses, 1H, 13C, and mass spectrometry.
- Discovered that serine amide alcohols (**4, 7a-9a**) are also active against prostate cell lines.
- Observed a structure activity relationship in both serine amides and N-Boc-serine amides with an increase in the activity as the alkyl chain length increases.

Reportable Outcomes

We are putting together and will submit an abstract to the National American Chemical Society meeting to be held in New Oleans, LA in Spring of 2003. The material to be presented is the data presented in this report.

Conclusions

In Year 1 we successfully completed the synthesis of the R and S optical isomers of SAP (14:0) by an improved synthetic sequence shown in Scheme 3. In the first year it took us some time to come up with this much-improved synthetic sequence. We have completed the synthesis of 10:0, 14:0, 18:0, and 19:0 D-SAPs shown in scheme 4. The structures of all new compounds synthesized were confirmed using elemental analysis and spectroscopic data (1H, 13C, IR and Mass Spectrometry).

We have tested for the affinity of the synthesized compounds in PC-3, DU-145, and LNCaP cell lines as we proposed earlier. In addition to these cell lines we have also tested for affinity of these compounds in two additional PPC-1 and TSU cell lines (data shown in Table 1). These new analogs have provided valuable insight as to the importance of chirality, lipid solubility, spatial orientation, and important functional groups of the pharamcophore and for the optimization of the antiproliferative actions of this new set of drugs. One of the key factors is that the Serine Amides and the N-BOC-Serine Amide alcohol series which lack a phosphate show higher activity with longer aliphatic chains. We have not found the optimum length of the aliphatic chain in these two series. In earliar studies it appeared in our Serine Amide Phosphate (SAP) series that the alphatic chain is optimum at C-14 on DU-145 and PC-3 cell lines. However, the pure isomers do not show any large differences and the activity is different from earliar tests run on the racemic mixtures. We have observed that one of the isomers of the SAP series with the aliphatic chain being C-18 does have moderate acitivity against all of the cancer

cell lines. Again it appears that we should make and test new SAP series with longer aliphatic chains and investigate the effects of unsaturation.

References

None

Appendix

None